

DIURNAL CHANGES IN THE ACCURACY OF THE HONEYBEE FORAGING RHYTHM

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ABSTRACT

The ability of honeybees to time foraging visits to an artificial nectar source was analyzed with respect to the time of day of food source presentation. A consistent regimen of orientation and training to the food source, which was available only during a specific one-hour period of the day for each particular experiment, allowed quantitative comparisons to be made among groups of bees trained at different feeding times. Bees trained early in the day showed the most precise time-keeping ability whereas those trained to midday or late afternoon food sources were significantly less accurate. In all of the experiments, the bees anticipated the onset of the training period, but the duration of the anticipatory component of the response was dependent upon the training time. Similarly, other parameters (coefficients of skewness and kurtosis and the shut-down in the number of arrivals immediately after the end of the training time) describing the distribution of foraging flights in time varied according to times in the diurnal cycle when the food source was offered. These results suggest that honeybees more efficiently exploit a daily floral nectar source if it is available early in the morning than if it is offered late in the day. Possible mechanisms underlying the observed differences in temporal orientation are discussed.

INTRODUCTION

The remarkable ability of honeybees to precisely associate the time of day with the presentation of a food reward was first described in the anecdotal reports of von Buttel-Reepen (1900) and Forel (1910) and later in the classical experiments of Beling (1929) and Wahl (1932). Because nectar secretion and pollen presentation of flowers are rhythmic in nature and offered only during specific time intervals of the day, the learned time-sense of honeybees is considered to be highly adaptive (Kleber, 1935). Experiments showing that honeybee foraging rhythms persist under strictly controlled constant conditions of light, temperature, and humidity (Beling, 1929; Wahl, 1932) provide evidence that the bees' ability to remember time is based on an endogenous clock mechanism, and is not driven by external periodic time cues. Unequivocal evidence of the endogenous nature of this biological clock was provided by Renner (1955, 1957) in a series of global translocation experiments. Bees trained to gather food at a fixed time of the day in a closed flight room, under constant illumination and temperature, returned to the food source about 24 hours after the last food reward despite having been displaced over several time zones overnight. However, when the experiments were repeated, not under constant conditions but in the open, the bees re-entrained to the new local time (Renner, 1959). Thus exogenous rhythmic cycles, such as the diurnal repetitions of light and dark, serve to re-set or synchronize the foraging rhythms to the natural environment.

Received 29 December 1982; accepted 25 March 1983.

Later work demonstrated that in the absence of such external time cues (Zeitgebers) honeybee foraging activity occurs with a periodicity of from 23.4 to 23.8 hours in constant illumination (Bennett and Renner, 1963; Beier, 1968; Beier and Lindauer, 1970; Moore, unpublished data) and therefore this rhythm is circadian (period about 24 hours).

The versatility of the honeybee foraging rhythm sets it apart from the activity rhythms of most other animals so far investigated. Honeybees can be trained to visit a food source at any time of the day (Beling, 1929; Wahl, 1932) and, in fact, have been trained to as many as nine discrete feeding times per day (Koltermann, 1974). Bees also learn the locations of widely separate sites and remember to visit them at the correct times (Wahl, 1932; Finke, 1958).

Because foraging bees can associate any time of the daily cycle with a food reward, the honeybee foraging rhythm must involve a "continuously consulted clock" (Pittendrigh, 1958), as do the biological clocks underlying time-compensated celestial orientation in a number of animals, including birds (Hoffmann, 1960; Sauer and Sauer, 1960), bees (von Frisch, 1950), and amphipod crustaceans (Pardi and Grassi, 1955). Despite the long tradition of work in this field, the mechanism of the foraging time-sense remains obscure. One basic question which has not previously been addressed by systematic experimentation is whether the foraging time-sense is equally punctual for all phases of the daylight cycle. That question is the subject of the present study.

MATERIALS AND METHODS

Fifteen field experiments were done, using a consistent training regimen and testing of the time-sense. The procedures used are very similar to the methods employed by von Frisch (review, 1967) for training honeybees to visit an artificial food source. First, worker honeybees were enticed out of the hive with 2 *M* sucrose solution applied to strips of filter paper which were scented with a drop of floral essential oil and placed at the hive entrance. Next, bees feeding from the sucrose were transported carefully on the filter paper strips to a small feeding table located approximately one meter from the hive entrance. This procedure of transporting the bees was repeated several times. A watchglass (6.25 cm in diameter) containing 2 *M* sucrose solution and resting upon a 15 cm white filter paper circle was placed in the center of the feeding table. It was kept filled with sucrose solution throughout the training period. One drop of the essential oil, which was used at the hive entrance, was applied via a pasteur pipette to four equidistant spots at the edge ("compass points") of the filter paper disc. After 10–15 bees had flown from the hive to land and feed at the watchglass, the feeding table was moved several meters farther from the hive. This procedure was repeated, allowing about five minutes of feeding at each location. The table was moved in gradually increasing steps until it was located 100 meters from the hive; the entire procedure was accomplished within one hour. During this time, more bees were constantly being recruited from the hive such that at the end of the hour as many as 100 bees or more were typically foraging at the watchglass. Toward the end of this one-hour "orientation" training, the sucrose solution was not replenished but allowed to dry up coincident with the termination of the training period. At this time, the scented filter paper disc was removed and the feeding table and the watchglass were thoroughly rinsed with water to remove all traces of sucrose. The table and watchglass were then quickly dried and the watchglass was re-positioned over a fresh unscented filter paper disc on the table. The feeding table was kept at this location for the remainder of the experiment.

During the three days following orientation, bees were trained at the same time of day as the orientation. Within the one-hour training period, the watchglass was kept supplied with sucrose and the filter paper was scented, as before. Again, the sucrose and scent were promptly removed at the termination of the one-hour training period, but the empty watchglass and unscented filter paper remained on the training table at all times other than the training period.

On the fifth day, designated the "test" day, the watchglass remained empty and the filter paper unscented to eliminate the possibility of any recruitment of bees from the hive due to the presence of sugar or odor clinging to returning foragers. The times of arrivals of bees at the watchglass were monitored from sunrise to sunset. Flights within about 15 cm of the watchglass as well as direct landings on it were scored as arrivals. Individual bees were not marked in these experiments, but because it took a minimum of about three minutes for a bee to make a round trip between hive and feeding station, it was fairly easy to keep track of individual bees once they arrived at the station. Although each bee may make many return visits (Beling, 1929; Wahl, 1932; Moore, Siegfried, Wilson, and Rankin, in preparation), they were not counted more than once per visit.

The experiments were designed to mimic the manner in which a foraging group naturally exploits a newly-found food source. The numbers of bees arriving at the feeding station were not restricted, and foragers were free to recruit their hive mates. On test days, the distribution of arrivals at the training station represented how the bees, as a group, incorporated the training time into their time-sense.

In one additional experiment, the training time was increased to three hours but otherwise conditions were identical to those outlined above. Lilac scent was used for orientation and training in this particular experiment.

Honeybees obtained locally from area beekeepers were used in all of the experiments. Although the bees were not of a pure strain, they were predominantly *Apis mellifera ligustica*. The experiments took place either at Balcones Research Center or Brackenridge Field Laboratory of the University of Texas, which are located within the city limits of Austin, Texas. All of the experiments were done on bright, clear to partly-cloudy, warm days.

The results are displayed on graphs showing the number of arrivals per time, in five-minute intervals, beginning with official sunrise or the first arrival at the feeding station (sometimes a forager would arrive just before official sunrise). Data from the individual experiments were combined into appropriate groups according to the time of day of training and the resulting frequency distributions were subdivided quantitatively into "accurate" and "inaccurate" arrivals. The mean arrival time and the coefficients of skewness and kurtosis were tested in each case by employing the *t*-distribution (Sokal and Rohlf, 1969). Comparisons for significant differences among the groups were made using chi-square analysis. Additionally, differences with respect to skewness and kurtosis from the combined data were compared using the *t*-test.

RESULTS

The foraging experiments were partitioned into three groups according to the time of day in which the training time took place. Training occurred between one and three hours after sunrise in the morning group (Fig. 1). The noon group was trained between five and seven hours after sunrise (Fig. 2), while the evening group was trained between eight and ten hours after sunrise (Fig. 3).

All groups tested anticipated the training time. On test days we observed a

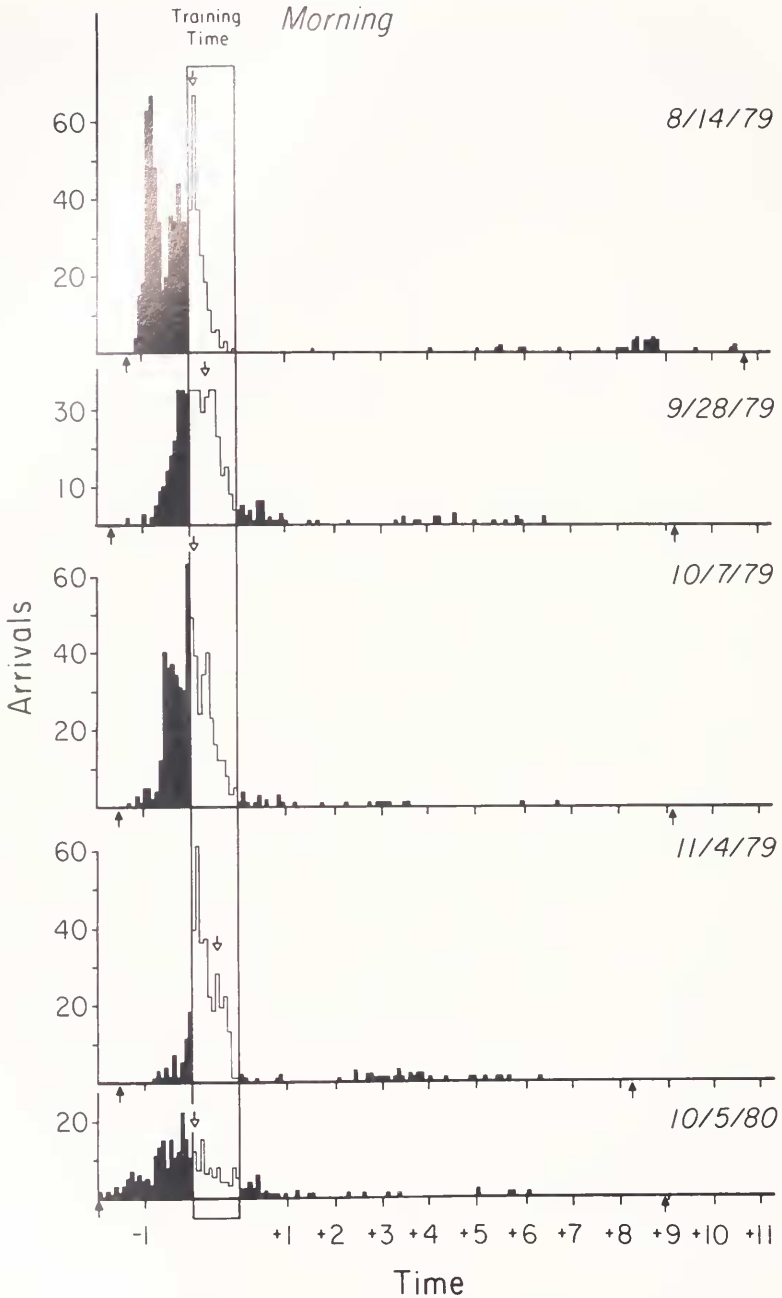


FIGURE 1. Morning group experiments. The distributions of arrivals on test days, at the feeding dish, are plotted in five-minute intervals with sunrise and sunset indicated by upward-pointing arrows. The number of hours preceding and following the training time (negative and positive values, respectively) are shown at the bottom of the figure. Framed within the vertically-extended rectangle are the one-hour training periods. The mean of each forager arrival distribution is indicated by a downward-pointing arrow above the response curve.

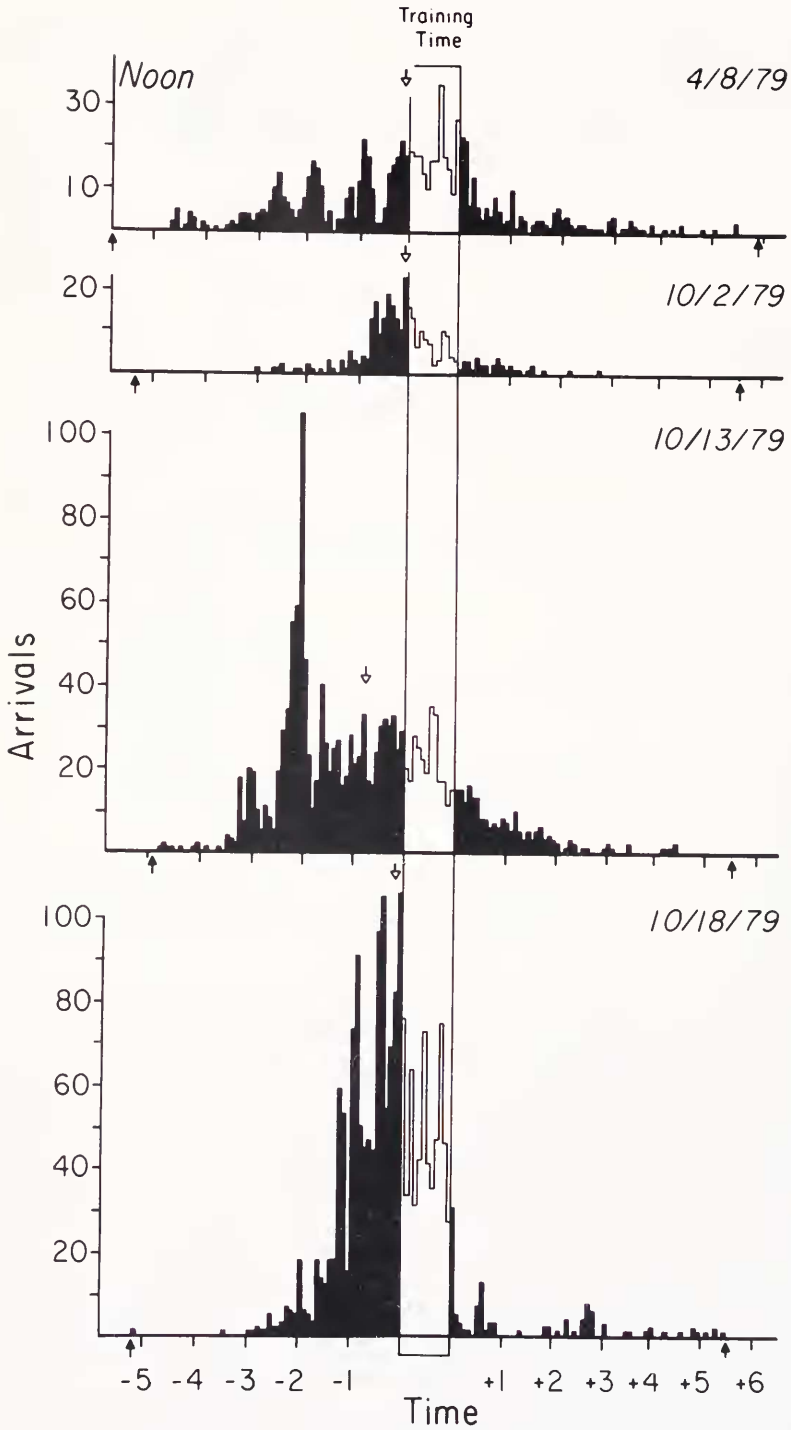


FIGURE 2. Noon group experiments. Details as in Figure 1.

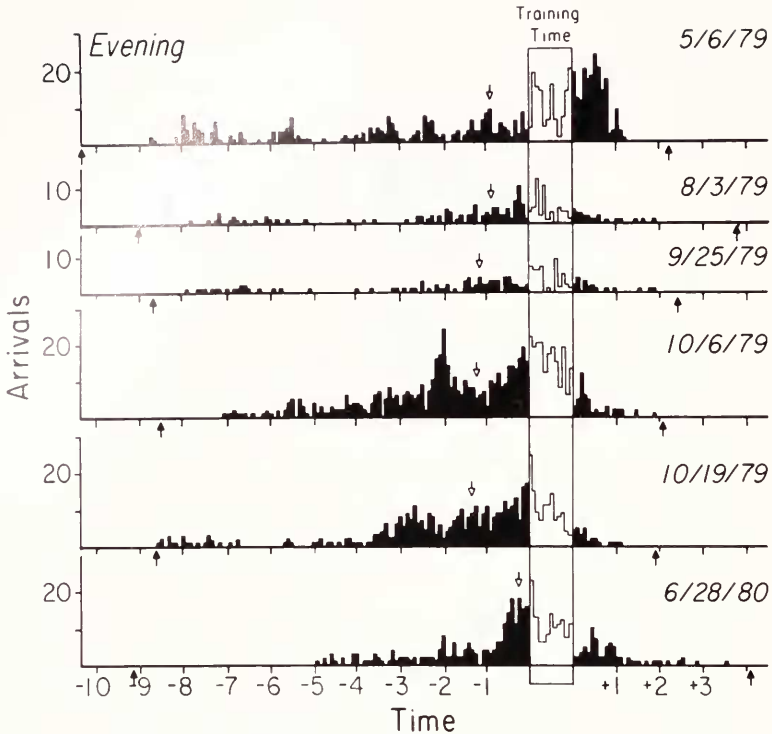


FIGURE 3. Evening group experiments. Details as in Figure 1.

considerable number of arrivals at the foraging station before the training time. This is consistent with the results reported previously (Beling, 1929; Wahl, 1932) and, according to von Frisch (1967), gives honeybees a competitive advantage over other nectar foragers and should not necessarily be construed as an inaccuracy in the foraging time-sense.

On test days, almost all experiments showed less bee activity at the end than at the beginning of the training period. This is to be expected if bees cue on the onset of the training period or, alternatively, if after several unrewarded flights to the food source at the "correct" time, the tendency to make any further return visits is lessened. The importance of the beginning of the training time was demonstrated by a morning experiment in which the training period was extended from one hour to three (Fig. 4). In this experiment, most of the activity (66.2%) occurred in the one hour immediately before and the first hour within the training period. By the end of the training period, the response level was reduced to only 6% of the value at the beginning.

Consistent with the anticipatory nature of the foraging response pattern, most experiments are characterized by fewer arrivals after the training period than before it. The three experimental groups differ markedly, however, in other parameters which describe the temporal distribution of their responses.

In terms of accuracy, or the precision of the foraging time-sense with respect to training time, it appears that the morning group was superior to both the noon and evening groups. To estimate forager accuracy, the total number of arrivals within the training time and within the one-hour period immediately prior to it are des-

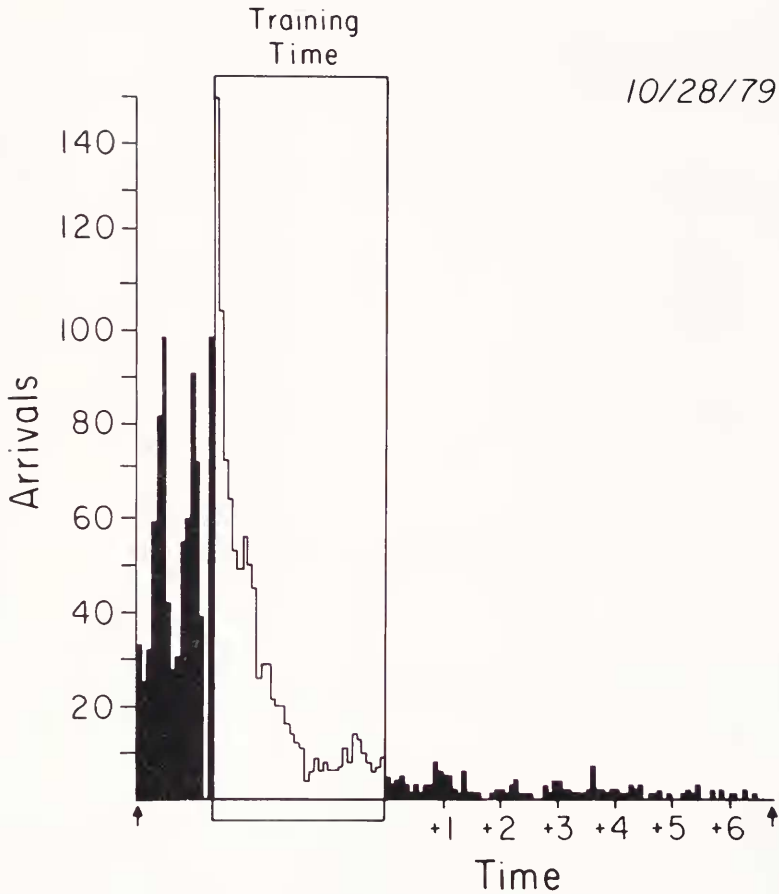


FIGURE 4. Morning group experiment with training duration of three hours. All other details as in Figure 1.

ignated as “accurate” and all arrivals at other times are considered “inaccurate.” This measure of time-sense precision assumes with von Frisch (1967) that anticipatory visits are an integral part of the foraging behavior of honeybees and should not be regarded as inaccurate. The average percentage of “accurate” arrivals was 88.0% for the morning, 61.1% for the noon, and 48.9% for the evening experiments (Note that Table I shows percentages for individual experiments). A chi-square analysis demonstrates that the morning group significantly outperformed both the noon and evening groups ($P < 0.001$ in both cases). Also, the noon group was significantly more “accurate” than the evening group ($P < 0.001$).

Another parameter which is important in terms of foraging efficiency is the ability to predict when a nectar source will no longer be available, *i.e.*, to shut down foraging activity at the appropriate time. Only 3.4% of the total number of morning arrivals occurred within the one-hour period immediately following the training time. By comparison, the noon and evening groups exhibited much higher levels of activity at the foraging station after the termination of the training time than did the morning group; 7.3% and 13.2% respectively, of the total responses lie within this time-frame. This result is surprising, especially so for the evening group, since

TABLE I

Data summary for the individual field experiments

Group	Experi- ment	Hive	Scent	Total number of arrivals	% Accu- rate	Skewness coefficient	Kurtosis coefficient	No. of 5-min intervals between mean & training time onset**
MORNING	8/14/79	A	Lavender	715	92.2	3.97	17.96	-2
	9/28/79	A	Lilac	551	88.4	3.64	18.32	-5
	10/07/79	B	Lilac	597	94.5	4.37	32.76	-1
	11/04/79	C	Lilac	388	89.9	3.00	11.69	-7
	10/05/80	E	Lilac	295	74.9	2.68	13.91	0
NOON	4/08/79	D	Lavender	770	49.0	0.25*	3.66	+1
	10/02/79	B	Jasmine	295	79.0	0.45*	4.82	+1
	10/13/79	C	Jasmine	1497	38.1	0.58	3.24	+9
	10/18/79	A	Jasmine	1855	78.3	1.67	10.21	+2
EVENING	5/06/79	D	Lavender	549	32.6	-1.22	3.31	+11
	8/03/79	A	None	166	54.2	-1.48	4.62	+11
	9/25/79	C	Lavender	143	50.3	-1.31	3.87	+14
	10/06/79	A	Lavender	706	47.0	-0.78	3.17	+15
	10/19/79	B	Lavender	509	48.5	-1.49	5.58	+16
	6/28/80	E	Jasmine	432	60.9	-0.62	3.89	+3

* All of the coefficients of skewness and kurtosis are statistically significant at $P < 0.001$ except those denoted by a single asterisk, which are significant at $P < 0.01$.

** Negative values indicate that mean occurs before training time onset; positive values indicate that mean occurs after training time onset.

compared to morning foragers, the evening bees have fewer foraging hours left in the day. From a chi-square analysis, the morning group is significantly more adept ($P < 0.001$) at shutting down its activity than is the noon group which, in turn, has significantly fewer arrivals during this period than does the evening group ($P < 0.001$).

Another measure which may be used to quantify the accuracy of a foraging rhythm is the difference between mean arrival time and the onset of the training time. On the average (Table I), experiments in the morning had mean arrival times only 15 minutes before training time onset. Noon experiments were similar in this regard with an average of 16.25 min. In the evening experiments, however, this difference was almost one full hour. It is interesting to note that the means for the morning experiments all occurred within the training period whereas those for the noon and evening experiments occurred before the training time.

Apart from accuracy, it is evident that the response profiles differ markedly among the three groups. The morning experiments (Fig. 1) have arrival distributions with sharper and higher peaks, whereas the noon (Fig. 2) and evening (Fig. 3) arrival distribution curves have broader peaks. The distribution curves of the groups may be quantified using coefficients of skewness and kurtosis (Table I), which characterize the distribution of the responses about their means (Sokal and Rohlf, 1969). All of the morning and noon experiments are significantly skewed to the left. Additionally, all of the distributions are significantly leptokurtic; that is, there are more arrivals near the mean and at the tails of each distribution than in the intermediate regions. When the results from the experiments within each group are combined and normalized with respect to training time (Fig. 5), it can be seen that the skewness coefficients of the three groups differ significantly from one another (t -test, P

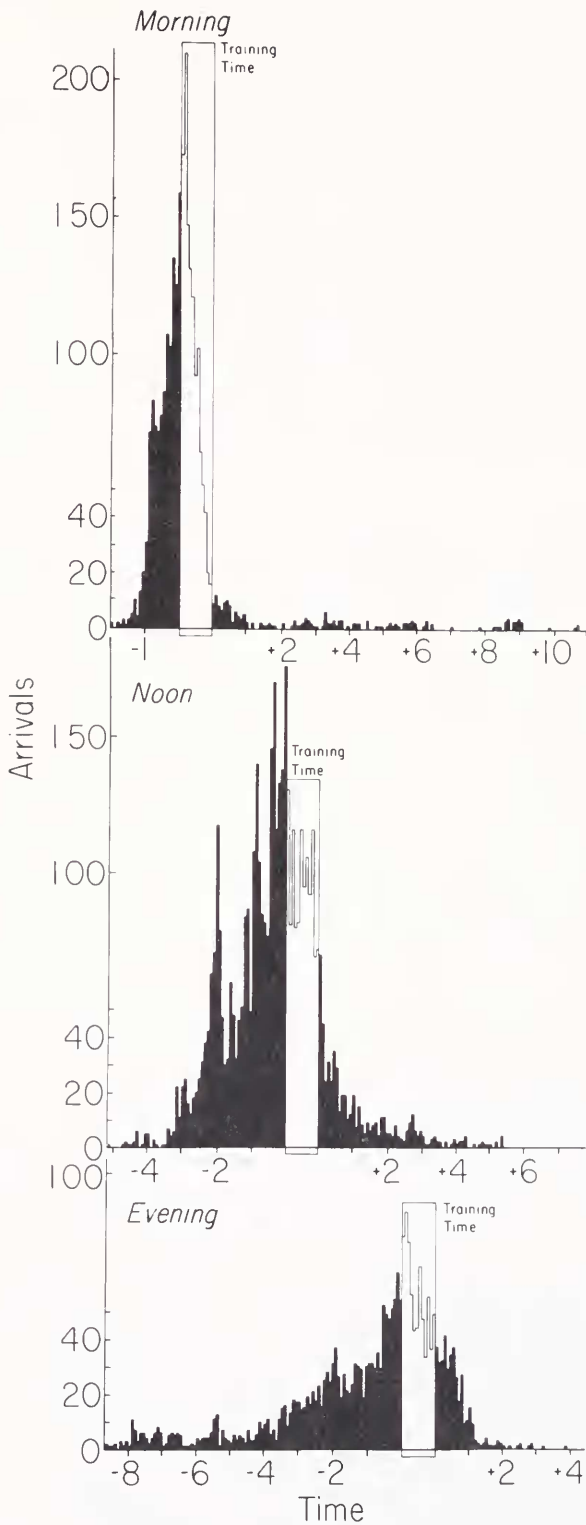


FIGURE 5. Combined data showing the results of all of the experiments within each training group (morning, noon, evening) normalized with respect to training time. Details as for Figures 1-3. Results illustrate the dependence of response profile on the time of day in which training occurs.

< 0.001). It may also be concluded that the morning group was significantly more leptokurtic than both the noon and evening groups (t -test, $P < 0.001$ in both cases) and the noon group was significantly more leptokurtic than the evening group ($P < 0.01$).

Three different scents were used in these experiments and in one experiment no scent was used. As expected, the time-dependent variability of the foraging rhythm was independent of the type of scent used during the orientation and training phases of the experiments (Table I). Comparisons of accuracy measurements, skewness, and kurtosis of individual experiments from the three training time groups (morning, noon, and evening) for the situations in which either the same scent or different scents were used, indicate a consistent pattern; there is a great deal of similarity within the same training group and a great deal of difference between groups.

DISCUSSION

The data reported here indicate that the time-keeping ability exhibited by foraging honeybees is not equal for all times of the day, but rather deteriorates somewhat from morning to evening. Morning training times are remembered with a much greater accuracy than late afternoon sessions, with midday training times intermediate between the two extremes. The observed differences in the accuracy of the honeybee time-sense persist over different times of the year (Table I) suggesting that the phenomenon is largely independent of changing conditions, including variations in natural flora. In experiments with individually marked bees, which will be reported separately (Moore *et al.*, in prep.), we have shown that all of the bees that visited the food dish on the test day of an afternoon experiment also did so on the first two training days (data on individual arrivals were not taken on the third training day). Thus there appears to be no differential recruitment of naive bees in the afternoon as opposed to the morning training groups. Similarly, judging from cuticle coloration, presence of body hairs, and over-all physical appearance, there appeared to be no difference in age distribution in morning as opposed to mid- or late-day foraging groups.

Why there should be a time-dependent variability in the foraging rhythm is not clear. The most energy-efficient strategy for the exploitation of a food source (which itself possesses a diurnal periodicity) by a honeybee colony would be to deploy a large number of foragers such that they arrive at the source just at the onset of nectar secretion (or experimentally, as the training time begins). This would ensure that a maximum number of bees would be able to forage for the entire duration of nectar presentation and, in turn, these bees could recruit a maximum number of new foragers during that time interval. Arrivals too early or too late at the food source must be considered to be energy-inefficient in this scheme. Early arrivals, or anticipation responses, are characteristic of all the experiments described in this study. Morning group experiments, however, show anticipation responses that we consider to be the most efficient, in that they are distributed such that the bulk of early arrivals occur just before the onset of the training time (beginning 15 to 60 minutes before training time). By comparison, midday and evening experiments are characterized by anticipation responses that may begin, at substantial levels, from three to six hours early. In addition, there are significantly fewer late arrivals for morning training times than for midday and evening training times.

There are two distinct possibilities that might account for time-dependent accuracy variation. Accuracy may be affected by signals from the environment which

may be used as secondary time cues for the initiation of foraging activity or alternatively, time-sense variation may be entirely endogenous in origin and completely independent of ongoing periodic external time cues. In both cases, the rhythm would be entrained to the natural day-night cycle, with the continuously-consulted program for the time-sense reset each day.

Considering the first possibility, time-dependent accuracy could perhaps be the result of external influences, such as social interactions within the hive (a social *Zeitgeber*). As the day progresses, there are likely to be frequent interactions occurring within the colony, such as waggle and round dances performed by returning foragers, recruitment of new foragers from the dances, and trophallactic contacts between foragers and hive bees. It might be expected that as food sources make themselves available throughout the day, the probability that a forager would interact with bees from different foraging groups increases. Such encounters presumably could induce a forager to be recruited to a new nectar source or to visit old sources more familiar to it. According to Körner (1939) and von Frisch (1940), foraging groups are spatially separated within the colony, with each group displaying a high degree of flower fidelity. Individual group members do not become active in the hive until the proper time; then they migrate toward the hive entrance and soon thereafter begin their foraging flights. However, there is some suggestion in the work of Medugorac and Lindauer (1967) of a social *Zeitgeber* influencing the honeybee time-sense. After CO₂-narcosis lasting several hours, bees visited a feeding place at their normal training time and again at a second time, delayed from the first by an amount proportional to the duration of the narcosis. If these narcotized bees were then transferred to another colony, which was trained to an altogether different feeding time, they exhibited three peaks of activity corresponding to the original training time, the narcosis-delayed time, and the host colony foraging time.

The alternative explanation, that the time-dependent variation in the accuracy of the foraging rhythm represents a true circadian phenomenon and thus reflects some inherent property of the endogenous clock(s) controlling this temporal behavior, is intriguing. There are at least three ways in which the endogenous control mechanism might produce the observed variation in precision. First, the clock itself may be less accurate later in the day. Second, the clock may be equally accurate at all times of the day but the coupling between the clock and foraging behavior may loosen as the day progresses. Thirdly, the clock may be accurate at all times but the coupling between foraging behavior and clock may be tight at all times but the behavior may be programmed to change from one part of the day to the next. The first two alternatives imply the existence of some imperfection in the foraging clock system that results in poorer foraging time accuracy later in the day. The last alternative implies that it may be advantageous to visit mid- and late-day nectar sources with greater time variance.

Whether there exists an adaptive reason for poorer temporal orientation later in the day is a difficult question to address. One might speculate that flower nectar rhythms may be less precise and hence less predictable as the day progresses, but little information is available on this point. Alternatively, perhaps the steady flow of early (anticipatory) visits to midday and evening sources is a programmed response to a greater incidence of competition from other nectar foragers. Indeed, it is perhaps more likely that the increased variance in mid- and late-day foraging behavior is adaptive than that there is a limit in the extent to which the clock system can respond to selection pressure for accuracy over the entire daily cycle.

The observations presented here provide the basis for future experimental anal-

yses of the honeybee time sense. Experiments to discriminate between exogenous and endogenous causes for accuracy variation in foraging are already in progress.

ACKNOWLEDGMENTS

The authors owe a large debt of gratitude to the many undergraduate observers that assisted us in this study. Without their enthusiastic participation this work certainly would not have been done. Special thanks go to Dr. R. H. Barth who helped coordinate this undertaking. We also thank H. D. Woodard, who was an integral part of this project during its early stages and Dr. R. H. Richardson and Ms. M. L. McAnelly for valuable suggestions with statistical analyses. We also thank Drs. W. J. Thompson, J. L. Larimer, D. Waller, and J. Rawlins for comments on an earlier version of the manuscript. This work was supported by NSF grant #BNS-7912105, University of Texas Research Institute grant #BRS 26-16941122 and NSF grant #PCM 8110568.

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